

Report for 2005PA41B: Fatty acid methyl ester (FAME) profiles of *Escherichia coli* and enterococci for predicting sources of microbial pollution

Publications

- Water Resources Research Institute Reports:
 - Duran, M., 2006, Fatty Acid Methyl Ester (FAME) Profiles of *Escherichia coli* and enterococci for Predicting Sources of Microbial Pollution, Pennsylvania Water Resources Research Center, University Park, PA, 5.
- Conference Proceedings:
 - Haznedaroğlu, B.Z. and M. Duran, 2005, Fatty Acid Methyl Ester (FAME) Profiles of Total Coliforms as a New Tool for Predicting Sources of Fecal Pollution, in Proceedings of the WEFTEC '05: The Water Quality Event, pp. 7142-7149.
 - Haznedaroğlu, B.Z. and M. Duran, 2005, Predicting Sources of Fecal Pollution Using Fatty Acid Methyl Ester (FAME) Profiling, in Proceedings of WEF TMDL 2005 Conference, pp. 634-644.
 - Duran, M. and B.Z. Haznedaroğlu, 2005, Host Specific Differences in Fatty Acid Methyl Ester Profiles of Indicator Organisms as a New Tool for Microbial Source Tracking, in Proceedings of the World Water and Environmental Resources Congress, ASCE Conference Proceedings, Vol. 173, Paper 316.
- Articles in Refereed Scientific Journals:
 - Haznedaroğlu, B.Z.; D.H. Zitomer; G.B. Hughes-Strange; M. Duran, 2005, Whole-cell Fatty Acid Composition of Total Coliforms to Predict Sources of Fecal Contamination, *Journal of Environmental Engineering*, ASCE, 131(10), 1426-1432.
- Dissertations:
 - Haznedaroğlu, B.Z., 2005, Fatty Acid Methyl Ester Profiling of Indicator Organisms for Microbial Source Tracking, "MS Thesis" Civil and Environmental Engineering Department, College of Engineering, Villanova University, Villanova, Pennsylvania, 93.
- Unclassified:
 - Duran, M.; B.Z. Haznedaroğlu; D.H. Zitomer, 2006, Microbial Source Tracking Using Host Specific FAME Profiles of Fecal Coliforms, *Water Research*, 40(1), 67–74.

Report Follows

INTRODUCTION

The Year 2000 National Water Quality Inventory lists microbial contamination as the leading cause of water quality impairment in rivers and streams in the US (EPA, 2000). The EPA report states that 93,000 miles of river and streams and 4,764 square miles of estuarine were contaminated with pathogens as of year 2000. According to the National Section 303(d) List, pathogens are the primary cause of water quality impairment nationwide, with 3,630 Total Maximum Daily Load (TMDL) being implemented due to presence of pathogens between 1996 and 2006 (EPA, 2006). Nine of these TMDL programs are in the state of Pennsylvania that is one of the significantly affected states.

Effective methods for predicting the sources of microbial pollution are needed to ensure timely and precise response to protect human and environmental health and to develop more accurate TMDL programs to maintain long-term water quality.

ABSTRACT

The overall objective of this study was to investigate the host-specific differences in fatty acid methyl ester (FAME) profiles of *Escherichia coli* and *enterococci* for the purpose of predicting sources of microbial pollution in water environments. In addition, commonly used indicator organisms total coliform (TC) and fecal coliform (FC) were included for the sake of completeness of the study. During the study period (January 1, 2005 through February 28, 2006), the FAME profiles of 303 TC, 314 FC, 605 *E. coli*, and 246 *enterococci* isolates were developed and the host specific differences in the FAME profiles were investigated using multivariate statistical tools. Due to a three month delay in project start, complete investigation of *enterococci* was not possible and thus the results from 246 *enterococci* isolates are not included in this report. The results indicate that the FAME profiles of TC, FC, and *E. coli* show statistically significant host-specific differences due to presence of signature FAMES and different relative abundance of common FAMES. The FAME profiles of FC isolates resulted in the most accurate source prediction, in comparison to those of TC and *E. coli*, with 95% precision in discriminating isolates of human origin from those of livestock and wildlife. The field testing showed that the signature FAMES are conserved in the aquatic environments and that known-source FC library predicted the dominant source of microbial pollution at 81% accuracy.

PRINCIPAL FINDINGS AND SIGNIFICANCE

1. The results of this study, funded by the PA Water Resources Center, strongly suggest the validity of the original hypothesis that FAME profiles of indicator organism show statistically significant host-specific differences.
 - The presence of three signature fatty acids in the isolates of human origin; 12:0 2OH, 12:0 3OH and 14:0 2OH, and other three in the isolates from the livestock samples; 15:0, 18:0 and 19:0 ISO, are the primary factors that allow accurate discrimination of the human isolates against the non-human sources when TC and FC are used as indicators.
 - In the case of *E. coli* isolates, the presence of fatty acids 10:0, 16:1 ω 5c, and 19:0 ISO suggest livestock sources fecal pollution. Furthermore, the absence of saturated fatty acid 18:0 implies that the likely source of the fecal pollution is non-human.

- In addition to presence of signature fatty acids, the differences in the average relative masses of some of the FAMES common in all host categories are statistically significant contributing to accurate classification of isolates when TC, FC, and *E. coli* are used as indicators.
2. While the FAME profiles of all of the indicator organisms resulted in comparable or higher accuracy when compared to other commonly used phenotypic methods, the results of this study suggest that using the FAME profiles of FC will result in more accurate source prediction than those of TC and *E. coli*. A linear discriminant function based on host specific differences in the FAME profiles of FC isolates classified the known-source isolates into three pooled host categories, human, livestock, and wildlife, with 95% accuracy.
 3. In the later stages of the study, the FAME profiles of FC isolated from environmental samples were also evaluated. The goal was to determine if the FAME profiles of the isolates from environmental samples can be discriminated using the known-source library constructed. The FAME profiles of the environmental isolates would provide insight regarding the conservation of known-source library FAMES in the environment and whether FAME profiling can be useful in microbial source tracking. A total of 5 water samples were also collected from a natural pond to culture environmental isolates. The pond provided a unique opportunity to field test the effectiveness of FAME profiling, since it was well-protected with the only apparent source of fecal pollution being waterfowl, particularly Canada geese (*Branta canadensis*), and perhaps to a smaller extent, other wildlife such as deer. The discriminant function developed from known-source FC library predicted that 81% of the total 37 FC isolates cultured from the pond were coming from wildlife sources.
 4. In conclusion, the results indicate that the FAME profiles of TC, FC, and *E. coli* isolates show host-specific differences and those differences can be used to differentiate the primary sources of the coliform isolates, which in turn can be used to predict the sources of fecal pollution in water environments.
 5. It is important to emphasize that since the FAME profiling relies on the presence of signature FAMES and differences in FAME profiles to identify primary host of the indicator organism, developing a large known-source libraries may not be necessary to predict sources of fecal pollution, as it is the case for the other phenotypic MST methods.
 6. Besides its accuracy, FAME profiling as an MST tool offers advantages such as being relatively rapid and highly economical (\$2.00-\$2.50 per isolate).

STUDENTS SUPPORTED

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2. Deniz Yurtsever '07, Water Resources and Environmental Engineering, MS
3. Kimberly L. Daileader '06, Civil and Environmental Engineering, BS
4. George B. Hughes-Strange, '06, Chemistry, BS
5. Jamie R. Lefkowitz '06, Civil and Environmental Engineering, BS

PRESENTATIONS AND OTHER INFORMATION TRANSFER ACTIVITIES

1. Duran, M. (Invited speaker), 2005. "Host Specific Differences in FAME Profiles of Fecal Coliforms as a New Tool to Predict Sources of Microbial Pollution", Presented at Center for Urban Environmental Research and Education, University of Maryland, Baltimore County, Baltimore, MD, April 1, 2005.
2. Results posted on Villanova Urban Stormwater Partnership (VUSP) website at: <http://www3.villanova.edu/VUSP>

AWARDS

None yet

REFERENCE

1. **U.S. Environmental Protection Agency (EPA)**. 2000. National water quality inventory: 1998 Report to Congress. EPA-841-R-00-001. 413 pp.
 2. **U. S. EPA National section 303(d) List**. 23 February 2006. [Online.]
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